

## REPRODUCTION IN THE SOCIAL BEES<sup>1</sup> (HYMENOPTERA: APIDAE)

WARWICK E. KERR,<sup>2</sup> RONALD ZUCCHI,<sup>3</sup>  
JULIO TAKESHI NAKADAIRA,<sup>4</sup> AND JOSÉ EDUARDO BUTOLO<sup>5</sup>  
UNIVERSITY OF SÃO PAULO, RIO CLARO, BRAZIL

RECEIVED FOR PUBLICATION JUNE 15, 1962

### ABSTRACT

Data are presented concerning the reproduction in Bombini, Apini and Meliponini.

1—*Melipona quadrifasciata*. All queens of this species mated only once and outside the hive. A queenless colony continues to kill virgin queens for almost 100 hours after being dequeened. Queens return from the mating flight with the male genitalia. A queen, marked on her return from her only mating flight, lived for three years and one month. Males average 1,156,850 spermatozoa. A recently mated queen had, in oviducts and spermatheca, 1,018,333 sperm. Since she returned with the male genitalia we concluded she mated with only one drone. Another queen which had laid only 30 eggs had 950,000 in the spermatheca indicating that about 97% of the sperm from the drone had reached the spermatheca: an efficiency of the ejaculation much greater than in *A. mellifera*. A spermatheca examined immediately after the queen returned from her mating flight showed spermatozoa moving at a speed of 11.4 to 16.0 mm per minute.

2—*Trigona (Tetragonisca) jaty*. Males gather in resting groups close to the entrance of the hive during swarming. These 3,000 males dispersed a few days after the queen was mated. More than one virgin flies from the mother colony to the new one during swarming. After a virgin is successfully mated remaining virgin queens are imprisoned in isolated waxy cells and die there. Spermatheca were found to contain 108,260 sperm.

3—*Trigona (Plebeia) droryana*. Males of this species, attracted by the

---

<sup>1</sup> This work was aided by the Conselho Nacional de Pesquisas (Brazil), the Rockefeller Foundation, C.A.P.E.S. and United States Dept. of Agriculture under Public Law 480.

<sup>2</sup> Professor of Biology in the Faculdade de Filosofia, Ciências e Letras de Rio Claro, State of São Paulo, Brazil.

<sup>3</sup> Instructor of Biology in the Faculdade de Filosofia, Ciências e Letras de Rio Claro, State of São Paulo, Brazil, under a graduate studentship of C.A.P.E.S.

<sup>4</sup> Actual address: Instituto Biológico, São Paulo, Brazil.

<sup>5</sup> Student of Faculdade de Filosofia, Ciências e Letras de Rio Claro.

smell of the orchid, *Trigonidium obtusum*, tried to mate with this flower. An anaesthetized queen placed on the flower (whose scent is believed to resemble that of the virgin queen) attracted males which tried to copulate with her in the normal position of solitary bees, male above female.

4—*Trigona (Scaptotrigona) postica*. During swarming as many as three virgin queens flew to the new home. A day later only one queen was found. A virgin queen born in a normal hive, but not mated, is tolerated for about 15 days, then she is persecuted by the bees and killed. Up to 8 virgin queens were found in a queen-right hive. Males leave the hive after a few days and then congregate in groups within 20–250 cm of the hive entrance. Greatest flight activity is between 1:00 and 3:00 P.M. Males feed directly from flowers within a flight range of about 600 meters, which is comparable to the flight range of workers. Males gathered in groups before a hive, may belong to several different colonies.

5—*Apis mellifera*. Sperm counts of male ejaculations varied from 1,600,000 to 9,610,000 with an average of about 6,000,000. No correlation was found between body weight of drones and sperm count. Drones from one hive lacked spermatozoa. A queen which had been laying for 40 days had 5,600,000 sperm in her spermatheca. Two queens dissected on their return from the mating flight showed 43,440,000 and 69,590,000 sperm in their oviducts and spermatheca suggesting matings with 7–12 drones on a single flight. Sperm in the spermatheca were actively motile for approximately 60 minutes before becoming non-motile. Their motility was slower than sperm of *M. quadrifasciata quadrifasciata*. There is a noticeable tendency, increasing as you move up the scale of social behavior, towards an increase in the genetically active population among social bees.

Most studies on reproduction of social bees have been made with the honey bee, *Apis mellifera* L. It was the common belief some 20 years ago that the queen of *Apis mellifera* mated on her nuptial flight with only one drone. Roberts (1944) (6), however, using strains of bees with genetic markers, showed that each queen in his apiary had copulated with at least two males. Triasko (1951, 1956), comparing the volume of spermatozoa in the ejaculate of a single drone with the volume found in the oviducts of the queen on her return from the nuptial flight, concluded that 7 males inseminated the queen on a single flight. Taber (1954), using genetic markers, also concluded that an average of 7 males mate with each queen. Taber and Wendel (1958) analyzing data from Taber, Peer and Cale, concluded that queens mate with 7 to 10 drones. Taber (1954) found the average duration of the nuptial flight to be 13 minutes.

Alber (1956) working with Sicilian bees, showed that queens mate only once during bad weather.

Peer (1957) obtained important data on the distance travelled,

by both queen and drone, during mating flights. His main conclusions are summarized in Table 1.

Table. 1. Data (Peer 1957) showing a decreasing percentage of fertilization with increasing distances of mating flights.

Distance between queens and apiary	Number of queens	Number of queens that began laying eggs			% of queens that began laying eggs
		15th day	23rd day	31st day	
In the apiary	9	9	—	—	100
5.1 km	9	8	—	—	89
9.8 km	9	6	—	—	67
12.9 km	12	—	3	2	42
16.3 km	12	—	—	3	25
18.3 km	11	—	—	—	0
22.5 km	11	—	—	—	0

Oertel (1956), by caging drones and then releasing them at various distances from their hives, showed that the percentage of returning drones decreased with increasing distances. Beyond 4 km no drones returned. It would seem, therefore, that the maximum flight range of queens would be approximately 12.3 km, that is 16.3 km (Table 1) minus 4 km travelled by the drone. This corresponds with the maximum range for bee flight found by Knaffl (1953).

Mackensen and Roberts (1947) found the average number of sperm in the spermathecae of queens to be 5,730,000 while drones average 9,000,000 sperm in the seminal vesicles.

In a detailed study of the evolution of behavior and of bionomics of the social bees it is necessary to make comparisons between honey bees and other social species. Bumblebees (*Bombini*) are the most primitive social *Apinae*. Fairchild and Barret (1906) observed *Bombus* (*Fervidobombus*) *fervidus* (Fabricius) mating on a tree trunk six inches above the ground. Frison (1927) obtained controlled mating within a glass dish in the species *B. (F.) americanorum* (Fabricius), *B. (Pyrobombus) bimaculatus* Cresson, and *B. (P.) vagans* F. Smith. Sladen (1912) suggests that *B. (Hortobombus) ruderatus* Fabricius mates in the open air.

Kerr and Krause (1950) observed that the queens of *Melipona quadrifasciata quadrifasciata* Lepeletier returns from her nuptial flight with the male genitalia attached. The exhaustive studies and research data of Nogueira Neto (1950, 1954) on swarming

in the stingless bees (*Meliponini*) have been available to us since 1949.

#### GENERAL METHODS

Direct observations have been made of the mating habits of several species of stingless bees and *Apis mellifera* L.

The Jaycox method (personal communication) of counting sperm has been used: 2 gr. of soluble starch is dissolved in 98 cc of double-distilled water. This solution can be used for two weeks if kept under refrigeration. The spermatheca is placed in 1 cc of the 2% starch solution and broken to release the sperm. A medicine dropper is used to homogenize the mixture and to put a drop in the Levy-Hauser haemocytometer. Counts of sperm are made rapidly under 400X magnification.

#### RESULTS

Results, and special methods are given for each species studied.

1. *Melipona quadrifasciata quadrifasciata* Lep. A queenless colony was placed in a Nogueira Neto hive (Nogueira Neto 1953) on a table inside the laboratory. A rubber tube 70 cm long and 3 cm in diameter led from the entrance of the hive through a hole in the glass window so bees will fly freely. This tube was cut 10 cm from the hive, and a piece of glass tube was used to connect the cut rubber parts so that every bee leaving or entering the hive could be seen. Observations were made continuously from 6 A.M. to 6 P.M.

**First experiment** . A *First Experiment* Jan. 27, 1958—A strong hive of *Melipona quadrifasciata quadrifasciata* was divided into two parts. The queenless part had ample brood and was used in our experiments.

Jan. 28, 1958—Bees began to work normally.

Jan. 29, 1958—The activity of workers was intense due to good weather. At 4 P.M. a virgin queen, killed by the workers was carried from the hive.

Jan. 30 and 31, 1958—Three more virgin queens were killed and carried out.

Feb. 1, 1958—A virgin queen left the hive. When she returned she was marked, clipped and released into the hive. She began laying in six days. (This queen lived until March 3, 1961, or for three years and one month).

The elimination of the virgin queens indicates that "queen substance" from the original queen was still present in the hive. The last virgin queen was killed 98 hours after the hive was divided and made queenless and the first virgin queen accepted by the colony was born 120 hours after the colony was made queenless. The accepted virgin queen was anaesthetized when she returned from her mating flight and the male genitalia were found in her genital tract thus confirming the observations of Kerr and Krause (1950). It was, therefore, concluded that queens of this species are inseminated only once.

**Second experiment** B *Second Experiment* Eighty-four hours after the queen had been killed in a colony, a virgin queen left the hive on her mating flight. She was dissected when she returned and the male genitalia were found attached. The spermatheca was removed, placed in the starch solution and examined microscopically, before the sperm were released. The sperm were moving like a dense cloud within the spermatheca. We could see that the cloud was formed by thousands of spermatozoa heads and made 10-14 complete turns per minute or at a speed of 11.4 to 16.0 mm per minute. This is a fantastic speed in comparison with the rate of movement in other animals. The sperm continued their movement for an hour and a half after the return from the nuptial flight when the spermatheca was ruptured and examined. The total number of sperm found in the mated queen two hours after the nuptial flight was 1,058,333 (standard deviation of  $\bar{x}$  65,000) being 985,000 found in the oviducts (standard deviation of  $\bar{x}$  65,000) and 33,333 (standard deviation of  $\bar{x}$  7,600) which had already entered the spermatheca. Another queen that had laid 30 eggs showed 950,000 sperm (standard deviation of  $\bar{x}$  55,000).

Two drones were dissected and their spermatozoa counted; the first produced 1,088,750 ( $\pm$  60,500) and the second 1,225,000 ( $\pm$  56,750). The number of sperm found in the drones and queens leads to the conclusion that queens are inseminated by only one male, confirming our suspicions when we saw the male genitalia inside the queen. So far as we could determine queens made only one mating flight.

If one touches the male genitalia while dissecting, ejaculation begins and proceeds in such a way that practically no sperm remain in the seminal vesicles. It seems probable that pressure,

when the genitalia of the drone enters the vagina of the queen, is the stimulus which causes ejaculation. The movement of the spermatozoa into the spermatheca is a point deserving comment. We found that 97% of the sperm deposited in the oviducts entered the spermatheca. This can only be possible if chemotaxis is the motivating force. The strong movements of the spermatozoa confirms this belief.

## 2. *Trigona (Tetragonisca) jaty* F. Smith

**Material** A young colony being formed by swarming, and an observation colony.

**Observations** No signs of swarming occurred until Oct. 3, 1959. A mated queen observed in the colony could still fly but had not started egg laying. Upon dissection 108,260 sperm were found in the spermatheca (Jaycox technique). She weighed 22.45 mg. On the same day we found a virgin queen imprisoned by workers among resin and wax.<sup>1</sup> There were no sperm in her spermatheca which suggests that virgin queens of *Trigona* go to the new colony before making their mating flight. Following the killing of the queens a new virgin left the mother colony and joined the swarm in the afternoon of Oct. 19, 1959. She mated on the same day and began laying a few days later.

During the period when queens were ready to mate an enormous group of about 3000 males stayed in front of the hive. After mating very few males remained even though about 30 males were still in front of the hive four days later.

On July 22, 1961 we again observed the beginning of swarming of a colony of *T. jaty*. Sixteen days later the new queen laid the first four eggs.

## 3. *Trigona (Plebeia) droryana* Friese

Kerr and Lopes (1962) were lucky in finding a species of orchid, *Trigonidium obtusum*, whose flowers attracted only males of *Trigona (Plebeia) droryana*. Tearing the flower apart they found that a small dark spot on the sepal was responsible for the attraction. The males tried desperately to copulate with that spot. When an old queen, anaesthetized with CO<sub>2</sub>, was placed on the sepal, males immediately tried to copulate with her in the nor-

---

<sup>1</sup> Dr. Paulo Nogueira-Neto informed us that detailed information on this behavior of *T. jaty* is reported in a paper of Mr. E. Juliani recently sent to press.

mal position used by all known solitary bees, that is, male above female.

4. *Trigona (Scaptotrigona) postica* Latreille.

Material and Methods: A colony of *Trigona (Scaptotrigona) postica* was used, and observations were done on swarming and on the behavior of males flying around the hive.

On July 20, 1961 about 10 workers of this species tried to invade a hive of *T. (Frieseomelitta) freiremaiai* Moure. On the following day one of these workers was observed placing communication marks (for communication in *T. (Scaptotrigona) postica* see Lindauer and Kerr, 1960). To avoid loss of the colony the hive was removed from its original location and returned five days later. However, on August 5th about 10–15 workers of *Trigona (Scaptotrigona) postica* began to again force their way into this weak colony. Three days later about 200 bees entered the hive and took possession of it, and a small group of males began to fly around it. On the morning of August 9 the swarm was examined and about 500 bees but no virgin queen was found. At 2:00 P.M. another examination was made and again no virgin queen was found. At 5:00 P.M. the hive was again inspected and a non-physogastric queen was found, showing that the queen arrived five days after swarming began. This queen was dissected but no sperm was found in either oviducts or spermatheca. On August 11th two active virgin queens were found in the hive but were not mutually antagonistic. The following day only one queen, slight larger than the day previous, was found and on August 20th she laid the first eggs.

Usually from 1–8 virgin queens may be found among the workers in hives of *T. postica*. Hebling, Kerr and Kerr (1962) observed a virgin queen from emergence to 17 days before she was killed by the workers.

Due to the significance of male behavior in reproduction and because *T. (Scaptotrigona) postica* hives provide an abundance of males throughout the year, it was decided to observe them. Usually these males congregate in groups resting quietly on branches, tree trunks, leaves, grass, etc, about 50–200 cm from the hive entrance. Colonies of *T. postica* always have large numbers of bees flying within a radius of 200 meters of the hive.

On April 16, 1960, a relatively cold day, samples of bees flying

around the hive entrance showed only workers. Males began to appear about 12:00 (noon) mostly in resting aggregations. At 1:00 P.M. we counted 265 resting males. Results from samples taken from flying bees after 1:00 P.M. are shown in Table 2. After 4:00 P.M. the number of resting males decreased to thirty, some of which made occasional flights.

Table 2. Samples of flying *Trigona postica* collected within 130 cm of the hive entrance.

Time	13.05	25	worker	25	males
	13.15	4	"	62	"
	14.22	6	"	17	"
	14.45	14	"	18	"
	15.10	3	"	21	"
	15.22	33	"	6	"
	15.55	17	"	16	"

Marking individual bees showed that when a male leaves the hive he stays with other resting males and flies very little. Length of flight periods increases with increasing age and reaches a maximum 10–15 days after the first flight is made. Males stayed in the swarm for various lengths of time. The maximum time a male remained alighted was 9 minutes.

A piece of wood placed close to the hive served as a locus of a group of males. Each day this rod was moved 20 cm further from the hive. Males continued to settle on the rod when it was 250 cm from the hive, but when we increased the distance to 270 cm no males came to it. We can, therefore, state that the maximum distance from the hive at which *T. postica* will establish a congregation spot is between 250 and 270 cm.

Very often wasps and honey bees were attracted to aggregations of *T. postica* and would lick the legs of the males of a given swarm. From 80 *postica* males washed in 6 cc of water we obtained 2.95% solids which shows that the males have some secretion on their bodies. The source of this substance is not known.

Of 136 males collected, 62 or 45% had large quantities of pollen on their bodies. Stingless bees, therefore, retain the primitive character of solitary bees in which males are able to earn their own living. In October 1960, a male of *T. (Scaptotrigona) postica* was observed taking nectar from a flower of *Senecio brasiliensis* Less. and in July 1961 a male of this species was found

collecting nectar in a flower of *Dombeya acutangula* Cav. We also observed that pollen collected by workers and drones are nearly always of the same species.

In a group of 800 males of *T. (Scaptotrigona) postica* there was a male of *T. (Scaptotrigona) bipunctata* (Lepeletier) from a hive 20 meters away. On another occasion two males *T. (Nannotrigona) testaceicornis* (Lepeletier) were found in a swarm of *T. postica* males. On March 30, 1962 a male of *Meliponula bocandei* (Spinola) (an African species) participated in a congregation of *T. postica* males for five days, and flew around the *postica* hive.

We believe that some kind of odor common to several species is responsible for these odd attractions. The number of males in an aggregate varies from a few to thousands.

On April 21, 1960 large numbers of *T. postica* drones were marked with pigment and released at various distances from the colony. Up to 600 meters males returned to the hive in great numbers but beyond 650 meters no drones returned. For this species, 600 meters is about the maximum range of flight for males; 680 meters is the maximum flight for workers of *T. postica*.

By marking drones from 4 hives we found that male aggregations contained drones from all 4 hives. This indicates that in this species panmixia is the rule.

##### 5. *Apis mellifera* L.

Method: Sperm counts were made on drones and queens of *Apis mellifera ligustica* and *Apis mellifera adansonii* both as a check on our experiments with the stingless bees and as a comparison with other data mentioned in the literature.

The males used were large adults collected at the hive entrance or returning from flight. They were pressed with the fingers and the product of the ejaculation was collected with a Mackensen and Roberts tip and syringe and immediately placed in a 2% starch solution. We believe the reason our counts are smaller than those of Roberts and Mackensen may be due to the amount of ejaculate which remained on the walls of the syringe and within the drone. However, we wanted to obtain counts from the spermatozoa which are ejaculated, and not from the ones which are found in the vesicles.

All drones were weighed before sperm counts were made.

Results: We found the average number of spermatozoa in drones to be 6,000,000 with a range of 1,600,000 to 9,610,000. One hive had males with no sperm at all. Since this is probably due to genetic factors the data are not included in the paper. We found no correlation between body weight and the number of sperm in adult drones.

Of seven queens examined, 4 returned virgin after the first mating flight. One queen, dissected after laying for 40 days, had 5,600,000 sperm in the spermatheca. A second queen dissected immediately after her return from a 15 minute mating flight had 190,000 sperm in the spermatheca, 69,400,000 in the oviducts or a total of 69,590,000 sperm. Since an average drone can supply 6,000,000 sperm, we suggest that this queen was inseminated by 11-12 drones. In a third queen, dissected after her second flight which had lasted 9 minutes, we found only the right oviduct full; not a single sperm was found in the left oviduct. There were 43,200,000 sperm in the right oviduct, 240,000 sperm in the spermatheca for a total of 43,440,000. This suggests she mated with 7 or 8 males. These data agree closely with the findings of Taber and Wendel (1958).

Observation of the spermatheca, under a Zeiss photo-microscope with episcopy attachments, showed that immediately after the nuptial flight the sperm are moving but not with the tremendous speed of *M. quadrifasciata* sperm. The velocity of *Apis* spermatozoa was estimated to be 0.05-0.07 mm per minute which is about 150 times slower than with *M. quadrifasciata*. One hour after the mating flight the sperm of *A. mellifera* were almost quiescent while in *M. quadrifasciata* the sperm were in continuous movement when the spermatheca was ruptured one hour and thirty minutes after the nuptial flight. Thus it would appear that the mechanism responsible by the migration of sperms to the spermatheca in *Apis mellifera* is not the same as in *Melipona*. A physical cause is suggested by these data.

#### GENERAL CONCLUSIONS

Since a female bee receives and stores, during a short mating period early in life, all of the sperm she will ever receive and because males are haploid and, barring mutations, produce only a single genetic type of sperm, it is evident that the greater the

number of males with which the queen mates in random matings the greater will be the genetically active population. Conversely, the greater the number of females which mate with a single male the smaller will be the genetically active population. From the data presented we can state that there is a tendency among social bees to increase the genetically active population. Males of solitary bees inseminate more than one female. Males of stingless bees (*Meliponini*) lose their genitalia during the mating act and therefore can inseminate only one female and virgin queens make only a single mating flight. Finally we have the *Apini* females that can rid themselves of the remaining male genitalia and can be inseminated by 7–12 males.

With increasing social organizations there is an increase in the capacity to store sperm.

Experiments on *Melipona* show that chemotaxis is the only acceptable theory to explain the migration of sperm into the spermatheca. The theory of chemotaxis, however, may not be valid for all species (*Apis mellifera* for instance).

#### Acknowledgments

The authors wish to express their thanks to Doctors Helbert R. Jaycox, Warren Whitcomb and P. Nogueira-Neto and Mr. S. Taber for many helpful suggestions.

#### Literature Cited

- Alber, M. A. 1956. Multiple mating. *Brit. Bee J.* **83**: 134–135; **84**: 6–7, 18–19.
- Fairchild, Davis and O. W. Barrett. 1906. Notes on the copulation of *Bombus fervidus*. *Proc. Ent. Soc. Washington*, **8**: 13–14, 1 pl.
- Frison, Theodore H. 1927. The fertilization and hibernation of queen bumblebees under controlled condition. *Jour. Ec. Ent.* **20**: 522–527.
- Hebling, N. J., Warwick E. Kerr and Florence Kerr. 1962. Divisão de trabalhos em *Trigona* (*Scaptotrigona*) *xanthotrycha* Moure. (In press).
- Kerr, W. E. and Wolfgang Krause. 1950. Contribuição para o conhecimento da bionomia dos Meliponini. *Dusenía* **1** (15): 275–282.
- Knaffl, H. 1953. Ueber die Flugweite und Entfernungen der Bienen. *Bienenforsch.* **2** (4): 131–140.
- Lindauer, M. and Warwick E. Kerr. 1960. Communication between the workers of stingless bees. *Bee World*, **41** (2): 29–41, **41** (3): 65–71.
- Mackensen, O. and W. E. Roberts. 1947. A manual for artificial insemination of queen bees. U.S.D.A. Division of Bee Culture.
- Nogueira-Neto, Paulo. 1953. A criação de abelhas indígenas sem ferrão. Ed. Chácaras e Quintais.

- . 1954. Notas bionômicas sobre Meliponíneos III—Sobre a euxeameagem (Hym. Apoidea). Arq. Mus. Nac. (Rio), 42: 419-452.
- Oertel, E. 1956. Observations on the flight of drone honey bees. Annals of the Ent. Soc. America 49 (5): 497-500.
- Peer, D. F. 1956. Multiple mating of queen honey bees. Jour. Econ. Ent., 49 No. 6. 741-743.
- . 1957. Further studies on the mating range of the honey bee, *Apis mellifera* L. The Canadian Entomologist 89 (3): 108-110.
- Robert, William C. 1944. Multiple mating of queen bees proved by progeny and flight tests. Gleanings in Bee Culture, 72 (6): 255-259, 303.
- Sladen, F. W. L. 1912. The bumble-bee, its life history and how to domesticate it. Mcmillan Co., London.
- Taber, S. 1954. The frequency of multiple matings of queen honey bees. Jour. Econ. Ent. 47 (6): 995-998.
- . and James Wendel. 1958. Concerning the number of times queen bees mate. Jour. Econ. Ent. 51 (6): 786-789.
- Triasko, V. V. 1951. Signais indicando a copula da rainha (In Russian). Pchelovodstvo, 11: 25-31 (Summary in Bee World 34: 18, 1953).
- . 1956. Repeated and multiple mating of queens. Pchelovodstvo, 33: 43-50 (in Russian).

#### ERRATUM

In Volume LXX No. 3, page 168 of the Journal of the New York Entomological Society the name of the first species of louse in the annotated list beginning on page 168, should have read:

*Enderleinellus longiceps* Kellog and Ferris  
instead of  
*Enderleinellus nitschi* Farenholz